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Alterations in Pulmonary Diffusing Capacity and Pulmonary Capillary Blood Volume with Negative Pressure Breathing *

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In 1915 Krogh first reported that the breath-holding pulmonary diffusing capacity for carbon monoxide (DL) is increased during exercise (1). Although change in the volume of blood in the pulmonary capillary bed (Vc), as determined by change in DL, can be produced by several mechanisms, it is during exercise that the greatest changes are observed. Yet, in a previous investigation in which some of the physiologic changes of exercise were simulated, such as alterations in alveolar ventilation, alveolar volume, cardiac output, mixed venous Pco₂, and mixed venous Po₂, there was little change in breath-holding DL (2).

Rosenberg and Forster (3), studying isolated cat lungs, concluded that the pressure across the walls of the pulmonary blood vessels is a primary factor in controlling the size of the pulmonary capillary bed as measured by DL. Observations by other investigators have agreed with this hypothesis (4). Breath-holding DL is increased by central venous engorgement with elevated pulmonary vascular pressure induced by pressure suit inflation (2, 5, 6). In addition, changes in gravitational orientation from the supine to erect position result in a small decrease in DL and Vc (7), which can be prevented by pressure suit inflation.

Head-down tilt increases right atrial pressure and also increases DL and Vc (8). Atropine diminishes central venous pressure (9, 10) and pulmonary blood volume and decreases DL and Vc in

the seated as well as the supine position. Positive pressure breathing has similar effects, and atropine and positive pressure breathing synergistically decrease breath-holding DL and Vc (11).

In view of these previous findings, it seemed likely that a negative intra-alveolar pressure should increase Vc and, therefore, DL by an increased filling of the capillaries resulting from the negative pressure, possibly due to an increase in effective distending pressure of the pulmonary capillary bed. Some previous studies, however, do not necessarily agree with this conclusion. Cotes, Snidal, and Shepard (12), in only two subjects, found variable effects of negative intra-alveolar pressure on DL and Vc. Arterial blood oxyhemoglobin desaturation occurs during negative pressure breathing (13), and an increase in DL has not been reported to occur concomitantly with a decrease in arterial blood oxygen saturation. During negative pressure breathing the mechanical properties of the lung and thorax are such that it has been equated to forward acceleration induced in a human centrifuge (radial acceleration vector from back to chest) (14). Significant arterial blood oxygen desaturation, incompletely corrected by oxygen therapy, also occurs during forward acceleration (15), and the pulmonary diffusing capacity is diminished (16). This analogy to forward acceleration makes it somewhat difficult to predict what might be the exact nature of the response of the pulmonary capillary bed to negative pressure breathing.

This investigation was designed to define more completely the effects of negative pressure breathing on the pulmonary capillary bed.

Methods

Subjects. Twelve previously trained, normal males 22 to 36 years old served as experimental subjects. They

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were in the postabsorptive state and rested 30 to 45 minutes before the experimental procedure. Three separate studies were required to complete the measurements of DL, V_c , and oxygen consumption at intra-alveolar pressures of -26 and -52 cm water. Not all the subjects completed the entire protocol, and replicate determinations were made in several.

Experimental conditions. All measurements were made with the subject in the seated position. During the last 5 minutes of the rest period the noseclip and mouthpiece were put in place. No effort was made to control respiratory frequency, tidal volume, or functional residual capacity. The subjects were carefully instructed to maintain an open glottis at all times.

Duplicate control measurements of breath-holding DL were made. The apparatus was then placed under reduced pressure, and the measurements were repeated at the end of 4 and 7 minutes of continuous full-phase negative pressure breathing and 3 minutes after termination of the 7-minute negative pressure breathing period. This time was chosen arbitrarily since DL is usually back to normal 3 minutes after termination of exercise. No attempt was made to follow the time course of the return of DL to normal after negative pressure breathing. The closed circuit apparatus consisted of a 50-gallon drum, connected to a vacuum source, in series with a CO_2 absorber and a Krogh respiratory valve. A Donald-

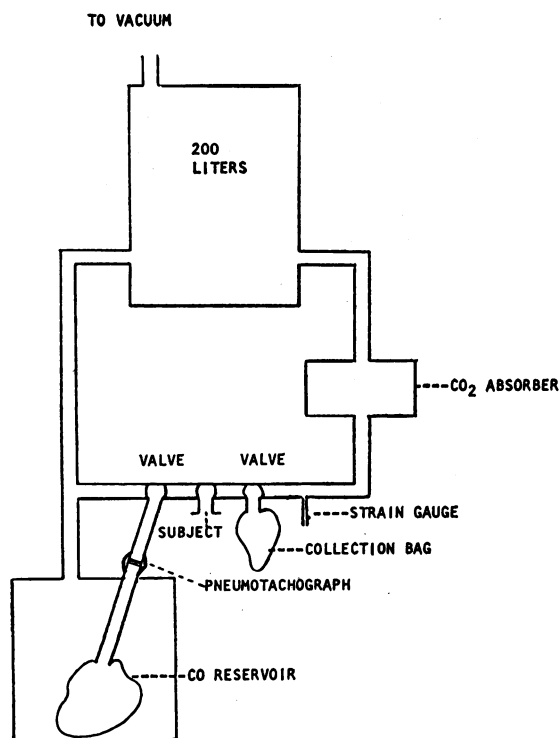


FIG. 1. APPARATUS USED TO MEASURE PULMONARY DIFFUSING CAPACITY (DL) AND INSTANTANEOUS PULMONARY CAPILLARY BLOOD VOLUME (V_c) DURING NEGATIVE PRESSURE BREATHING.

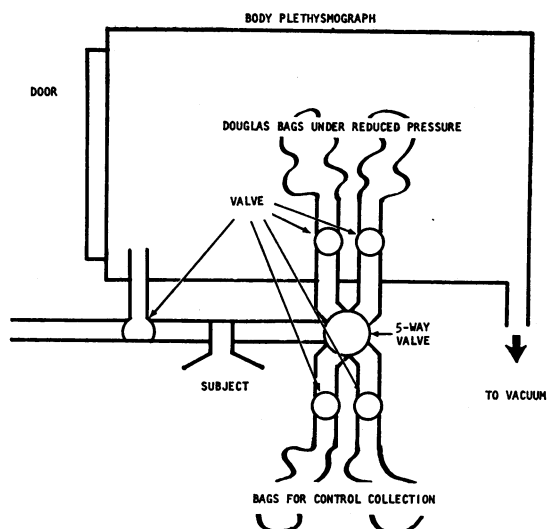


FIG. 2. OPEN CIRCUIT APPARATUS USED TO MEASURE OXYGEN CONSUMPTION DURING NEGATIVE PRESSURE BREATHING.

Christie box containing the carbon monoxide, neon, oxygen mixture was connected in parallel so that it was also under reduced pressure during the experimental procedure, as shown in Figure 1. The beginning of inspiration during the breath-holding maneuver, breath-holding time, and the inspiratory volume was determined with a calibrated pneumotachograph coupled to an integrating amplifier and electronically recorded. The beginning of expiration was defined from the output of a pressure gauge inserted into the expiratory line. This entire procedure was carried out in every subject with a pressure of -26 cm H_2O and with 0.4% CO, 1.0% neon, 21% O_2 in nitrogen as the inspired gas mixture used to determine DL. The procedure was repeated at -52 cm H_2O pressure with the same gas mixture. For calculation of V_c in five subjects, the procedure was repeated at -26 cm H_2O pressure with 0.4% CO and 1.0% neon in oxygen as the gas mixture for DL determination, and in four of these subjects, it was repeated at -52 cm H_2O pressure with the same gas mixture.

Oxygen consumption was measured by an open-system technique. A body plethysmograph was used as the negative transpulmonary pressure reservoir and was adjusted to proper pressure by a vacuum pump. The subject was at ambient pressure outside of the chamber and was connected to it through a carefully sealed Krogh valve and a five-way Rudolph valve by low resistance respiratory tubing that passed through the chamber wall by means of metal nipples. The expiratory side re-entered the box and was connected to 100-L Douglas bags as shown in Figure 2. Duplicate control measurements were made over 3-minute collection periods, and 5 minutes was allowed between these measurements. During the 7-minute period of pressure breathing, expired air was collected in one bag for 4 minutes and then in a second bag for the last 3 minutes. At the end of an

experimental period all expired gas was collected for the next 9 minutes in three equal periods. Ventilatory rate was monitored by a pneumograph and was recorded electronically. At least 30 minutes was allowed for recovery between all procedures.

Calculation of breath-holding diffusing capacity and pulmonary capillary blood volume. DL was determined by the Krogh breath-holding technique as modified by Ogilvie, Forster, Blakemore, and Morton (17) and as previously reported from this laboratory using a gas chromatograph to analyze alveolar samples for neon, carbon monoxide, carbon dioxide, and oxygen concentrations (18). The alveolar volume (V_A) was calculated from inspired and expired alveolar neon concentrations (N_{ei} , N_{ea}) and inspired volume (V_I).

$$V_A = \frac{N_{ei}}{N_{ea}} \times V_{IATPS}$$

$V_{ASTPD} = V_{AATPS} \times$ temperature correction factor. (ATPS is ambient temperature and pressure, saturated with water; STPD is standard temperature and pressure, dry.) The dead space of the apparatus used for both control and negative pressure measurements was constant, less than 100 ml, and was included in the calculation of V_A . In the formula for calculation of DL,

DL (milliliters CO_{STPD} per minute per millimeter Hg CO)

$$= \frac{(V_A)(60)}{(t)(P_B - 47)} \times \ln \frac{F_{ACO_0}}{F_{ACO_t}}$$

(where P_B is barometric pressure, t is time in seconds, and F_{ACO_0} and F_{ACO_t} are the initial and final CO concentrations in the expired alveolar sample), correction was made for pressure during negative pressure breathing as follows: at -26 cm water ($P_b-20-47$), and at -52 cm water ($P_b-40-47$).

V_c was calculated by the method of Roughton and Forster (19) using measurements of DL at two different alveolar O_2 tensions under each condition and assuming 2.5 as the ratio of permeability of the red cell membrane

to that of its interior. The absolute alveolar O_2 tension at which each determination was made was known from alveolar sample analysis. Mean capillary O_2 tension was then estimated according to the method of McNeill, Rankin, and Forster (20). θ for the appropriate O_2 tension was obtained from the data of Roughton, Forster, and Cander (21). The values for θ were calculated with an assumed CO capacity of 20 ml per 100 ml blood. Since each subject served as his own control and O_2 capacity is not greatly altered during negative pressure breathing (12), no correction was made for variations in CO capacity.

The oxygen cost of the added work of negative pressure breathing. Oxygen consumption and carbon dioxide excretion were measured as previously outlined with an open-circuit collecting system. The fractional concentrations of oxygen and carbon dioxide in expired air were measured, respectively, in a paramagnetic oxygen analyzer and an infrared carbon dioxide analyzer. The volume of expired air was measured in a gas meter. The volume inspired was calculated from the expired volume as follows:

$$V_I = V_E \frac{1 - F_{EO_2} - F_{ECO_2}}{1 - F_{IO_2}}$$

where V_I and V_E are inspired and expired volumes and F_{EO_2} , F_{ECO_2} , and F_{IO_2} are the fractional concentrations of oxygen and carbon dioxide in expired and inspired air. Oxygen consumption and carbon dioxide excretion were calculated in the usual manner and are reported at STPD. Minute ventilation and tidal volume are reported at BTPS (body temperature and pressure, saturated with water) with appropriate correction for the difference in gas volumes resulting from the reduction in ambient pressure during the experimental breathing periods. The additional oxygen consumed during pressure breathing was determined by assuming that the pre-experimental control oxygen consumption represented the base-line value for the subsequent period.

TABLE I

Pulmonary diffusing capacity (DL) and alveolar volume (V_A) during negative pressure breathing

	-26 cm								-52 cm							
	Control		4 minutes		7 minutes		Post 3 minutes		Control		4 minutes		7 minutes		Post 3 minutes	
	DL*	V_A †	DL	V_A	DL	V_A	DL	V_A	DL	V_A	DL	V_A	DL	V_A	DL	V_A
S.S.	31.1	4.42	45.5	5.44	49	5.03	36.3	5.28	31.1	4.42	46.5	4.94	37.5	4.23	36.8	4.33
R.D.	43.2	3.85	54.0	5.27	74.8	5.48	40.7	4.71	43.9	4.41	42.7	3.71	49.0	3.63	50.4	4.61
J.R.	38.8	5.97	50.7	6.64	46.9	6.95	39.2	6.19	36.7	5.86	45.9	6.41	41.3	6.41	43.2	6.92
C.D.	30.1	5.34	39.5	5.70	34.9	5.54	31.7	5.86	31.4	5.58	44.1	4.99	41.8	4.80	35.4	5.97
N.V.R.	39.4	5.05	51.5	5.30	69.2	5.50	57.0	4.90	32.1	4.80	41.8	3.05	36.6	3.56	34.5	3.38
M.L.	43.7	5.42	57.0	5.95	49.8	5.38	38.4	4.99	39.0	4.15	59.7	5.09	65.9	5.06	45.2	5.58
J.O.	30.9	4.36	37.6	4.65	32.1	4.58	34.1	5.10	28.4	3.96	53.1	4.46	50.6	4.29	27.3	4.26
L.D.	37.8	6.48	43.6	7.04	41.0	6.80	37.1	6.91								
Mean	36.9	5.11	47.4	5.75	49.7	5.66	39.2	5.49	34.6	4.74	48.2	4.66	46.2	4.86	39.0	4.98
SD	± 5.2	± 0.82	± 6.5	± 0.73	± 11.4	± 0.76	± 7.2	± 0.71	± 5.0	± 0.67	± 5.9	± 0.98	± 9.4	± 0.94	± 7.2	± 1.07
p			<.01	<.01	<.005	<.05	NS	NS			<.005	NS	<.005	NS	<.025	NS

* DL = milliliters CO per minute per millimeter Hg.

† V_A = liters per breath.

TABLE II
Pulmonary capillary blood volume (V_c) during negative pressure breathing

	-26 cm				-52 cm			
	Control	4 minutes	7 minutes	Post 3 minutes	Control	4 minutes	7 minutes	Post 3 minutes
	<i>ml</i>				<i>ml</i>			
S.S.	77	97	118	87	89	100	102	79
R.D.	100	90	90	94	96	100	108	104
N.V.R.	98	143	143	110	110	157	125	91
J.O.	68	79	93	68	72	94	96	80
L.D.	120	130	135	115				
Mean	93	99	116	95	92	113	108	88
SD	±18	±19	±21	±17	±14	±26	±11	±10
p		NS	<.05	NS		<.01	<.05	NS

Statistical analysis. The data were analyzed by the method of paired comparisons with the Student *t* test, each subject serving as his own control (22). Significance was determined at the 95% confidence level.

Results

Pulmonary diffusing capacity and capillary blood volume. DL was increased significantly after 4 and 7 minutes of continuous negative pressure breathing, both -26 cm H₂O and -52 cm H₂O, and returned to prepressure breathing control levels within 3 minutes after terminating -26 cm H₂O pressure breathing, but remained slightly elevated 3 minutes after terminating -52 cm H₂O pressure breathing (Table I). The differences in DL between 4 and 7 minutes and -26 and -52 cm H₂O are not significant. VA was increased

slightly during negative pressure breathing at -26 cm H₂O, but not enough to be a factor in causing the marked increase in DL. The reason for the increase in VA is not known.

After 4 minutes of breathing against -26 cm H₂O continuous negative pressure, the observed increase in V_c was not significant. However, at 7 minutes V_c was significantly increased from the control level (Table II). With -52 cm H₂O breathing, V_c was significantly increased at 4 minutes and at 7 minutes. V_c had returned to control levels within 3 minutes after negative pressure breathing.

Oxygen cost of negative pressure breathing. The exercise equivalent level of negative pressure breathing was assessed by the extent to which oxygen consumption increased. The results ob-

TABLE III
Oxygen consumption ($\dot{V}O_2$), carbon dioxide excretion ($\dot{V}CO_2$), the respiratory exchange ratio (RE), and minute ventilation (\dot{V}_E) and tidal volume (\dot{V}_T) during negative pressure breathing

	-26 cm			-52 cm		
	Control 1	Control 2	5-7 minutes	Control 1	Control 2	5-7 minutes
$\dot{V}O_2$ (ml_{STPD})	287	276	311	304	298	348
SD	±37.9	±46.6	±35.2	±38.4	±32.3	±34.6
p		NS	<.025		NS	<.01
$\dot{V}CO_2$ (ml_{STPD})	248	253	309	253	257	334
SD	±30.7	±61.0	±78.6	±48.3	±52	±86
p		NS	<.01		NS	<.005
RE	.87	.92	.98	.83	.85	.94
SD	±.05	±.13	±.15	±.08	±.12	±.17
p		NS	<.025		NS	<.01
\dot{V}_E (L_{BTFS})	8.88	8.83	12.02	8.66	8.87	11.45
SD	±2.6	±2.8	±3.6	±2.02	±1.8	±3.7
p		NS	<.05		NS	<.05
\dot{V}_T (ml_{BTFS})	792	800	1,158	848	853	1,261
SD	±340	±372	±617	±302	±314	±477
p		NS	<.005		NS	<.05

tained for duplicate control measurements, shown in Table III, were averaged, since measurements taken 3 minutes apart were not statistically different. The additional oxygen consumed was statistically significant.

Discussion

This study gives further information about how the size of the pulmonary capillary bed can be changed. Continuous negative pressure breathing consistently increased breath-holding DL and Vc to values comparable to those demonstrated during moderately severe exercise (23). It seems reasonable that this increase should occur. Although there are some conflicting observations (24), most investigators have found an increase in thoracic blood volume during negative pressure breathing (25-27). Ernsting (26) reported a decrease in lung compliance during negative pressure breathing, which could be due to the increased amount of blood in the lung.

Unlike exercise, however, a substantial increase in energy expenditure does not occur, indicating that the increase in DL and Vc during negative pressure breathing is not due to the metabolic effects of muscular work.

DL increases as O₂ tension decreases because of the change in the reaction rate of CO with hemoglobin (19). Negative intra-alveolar pressure can be expected to slightly diminish alveolar oxygen tension, and the decrease in peripheral arterial oxyhemoglobin saturation in man at - 52 cm water reported by Steiner and Behnke (13) is small (from 96.9% to 92.6% saturation). The associated decrease in tension is not sufficiently great to account for the large increase in DL.

Mean alveolar volume at which DL was measured was significantly increased after 4 and 7 minutes of - 26 cm H₂O breathing. This is likely due to the difference in inspiratory effort between normal breathing and negative pressure breathing. Subjects were not required to take an absolutely maximal inspiration for DL measurement. The difference between mean control V_A and mean V_A at 4 minutes of - 26 cm H₂O breathing was .64 L and was the greatest increase observed. DL does increase when the V_A at which it is measured increases, but in a previous study (28), a 40% increase in V_A was associated with a 20% in-

crease in DL. The small increase in V_A in the present study, then, is not sufficient to be a factor in causing the large increase in DL observed. In addition, Ross and co-workers (2, 29) have reported that there is no significant effect upon DL of hyperventilation at rest or of controlled ventilation during exercise.

The findings in this study indicate that the increase in DL that occurs with negative pressure breathing is attributable to an increase in the size of the effectively ventilated pulmonary capillary bed (Vc). As postulated for exercise, the increase in Vc may be due to distention of already open capillaries or opening of capillaries previously closed. Other investigators (12) have suggested that these changes might be due to an increased blood flow through the lungs, to an increased filling of the capillaries resulting from the negative pressure, or to an increased pressure gradient between the alveolar capillaries and the left atrium. It is conceivable that through any one of these three mechanisms, alterations in the distribution of perfusion could occur, leading to increase in the size of the effective pulmonary capillary bed by the addition of capillaries not previously perfused or ventilated. On the other hand, either of these mechanisms might also produce an increase in the size of the previously open capillaries.

Other investigators (24, 25, 30) have shown that pulmonary blood flow is significantly increased during negative pressure breathing and, in the lung, upper zone perfusion is increased as blood flow goes up (31). Increasing cardiac output by techniques other than exercise, however, does not increase DL or, presumably, Vc (2, 32). This strongly suggests that the per se increase in pulmonary blood flow is not the mechanism by which DL and Vc are increased.

Normally, pulmonary vascular pressure is inadequate to sustain the necessary hydrostatic column to perfuse the apical zone in upright man (31, 33). During negative pressure breathing the observed increases in DL and Vc are considerably greater than can be accounted for solely by the abolition of the postural blood flow gradient (7). The recent report by West and Dollery (34) concerning the lack of a critical closing pressure phenomenon (35) in the pulmonary vascular bed indicates that perfusion bears a direct relation to the

effective distending pressure. Holt (36), Ting, Hong, and Rahn (37), and Lenfant and Howell (30) reported that although central venous pressures decrease with negative pressure breathing, the effective distending pressures are considerably increased, since the vascular pressure drop is only about one-half of the fall in applied alveolar pressure. West, Dollery, and Naimark (38) using the clearance rate of oxygen¹⁵-labeled CO₂ and Rosenberg and Forster (3) have concluded that the pressure across the walls of the pulmonary blood vessels is a primary factor in control of the size of the pulmonary capillary bed. Although the increase in V_c during negative pressure breathing must be due, at least in part, to more effective distribution of perfusion, it also seems certain that this is not the only way in which V_c is increased and that previously open capillaries are also being distended, probably as a mechanical consequence of the applied negative pressure.

Either some perfused alveoli also become less well ventilated, or direct capillary shunts are opened by the negative pressure, since a decrease in arterial oxygen saturation occurs during negative pressure breathing (13). In the dog, at least, the decreased arterial oxygen saturation during negative pressure breathing is not fully corrected by oxygen breathing, indicating the existence of direct pulmonary arteriovenous communications (30).

The findings of this study are interesting and important. The increases in DL reported here during negative pressure breathing are greater than have been produced by any technique other than active exercise. In a previous study (11), it was shown that DL and V_c are dependent on pulmonary vascular pressures, or volume, or both, and relatively independent of cardiac output. Positive pressure breathing decreases DL and V_c (11), but the changes in DL and V_c in the opposite direction during negative pressure breathing are of greater magnitude. This study gives further support to the concept that V_c is dependent on effective distending pressure and relatively independent of cardiac output.

There is a limit, however, to the relationship between distending pressure and the size of the effective pulmonary capillary bed. In this study, DL and V_c were not increased further with -52 cm H₂O pressure breathing in comparison with -26

cm H₂O pressure breathing. This agrees with the findings of a previous study in which the effective distending pressure was changed by increasing the central vascular pressure by head-down tilt or pressure suit inflation or both (8). Maximal DL increase occurred with an increase in right atrial pressure of about 7 mm Hg and did not increase further despite large increases in central vascular pressure.

Since the increase in DL observed during negative pressure breathing is more comparable to that occurring with exercise than under any other circumstance, the question naturally arises as to whether negative intra-alveolar pressure plays any part in the increased DL and V_c during exercise or is associated with the breath-holding maneuver and responsible, at least in part, for DL determined by the breath-holding technique being higher than DL measured by the steady-state technique. Answering these questions is beyond the scope of this paper. It should be pointed out, however, that Mostyn and his colleagues (39) found that a 7-second maximal breath-holding maneuver (similar to that required for measurement of breath-holding DL) during the measurement of steady-state DL with continuous exercise caused a further increase in DL and V_c. Intra-esophageal pressures were also measured and ranged from +12 to -20 cm H₂O during the held-breath maneuver. They concluded that the degree of lung inflation, not the pressures, during the held-breath maneuver accounted for the further increase in DL during exercise. Nevertheless, if their data for maximal values of inspiratory pressures during the inspiratory phase preceding the onset of breath holding (-14 to -100 cm H₂O) are plotted against the steady-state measurement for DL in each subject, the result is a linear regression equation ($y = .44x + 27.2$; x = esophageal pressure in centimeters H₂O, and y = DL in milliliters CO per minute per millimeter Hg) and a correlation coefficient of +.96. This definitely suggests that the mechanism may play some part in the difference between steady-state and breath-holding DL measurements and in the increase of DL during exercise.

Summary

Negative pressure breathing increases pulmonary capillary blood volume (V_c) and breath-hold-

ing diffusing capacity for carbon monoxide (DL) to a degree comparable to that occurring with moderate exercise. Oxygen consumption is not similarly increased, indicating that the increase is not related to the metabolic effects of the muscular activity. The findings indicate that the increase in DL with negative pressure breathing is attributable to an increase in the size of the effectively ventilated pulmonary capillary bed.

The increase in Vc during negative pressure breathing must be due, at least in part, to better distribution of perfusion, but it seems certain that previously open capillaries are also being distended, probably as a mechanical consequence of the applied negative pressure. This does not exclude the possibility that alterations in ventilation account for the previously described decrease in arterial oxygen saturation during negative pressure breathing.

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